

Attorney Docket No.: 12917 (PTQ-0027)
Inventors: Van Eyk et al.
Serial No.: 09/115,589
Filing Date: July 15, 1998
Page 2

page 30, line 3 with the following:

Western blot analysis was done according to Van Eyk et al.
1998 (*Circ. Res.* 82:261-71) or else the primary antibodies were
detected with goat anti-mouse IgG conjugated to alkaline
phosphatase (Jandel Scientific) and CDP-Star chemiluminescence
reagent (NEN-Mandel). The monoclonal antibodies used were anti-
TnT clone JLT-12 (Sigma Chemical Co., St Louis, Mo), anti- α -
actinin clone EA-53, (Sigma) or anti- α -actinin clone 157
(provided by Spectral Diagnostics, Toronto, Canada), anti-MLC1
(provided by Abbott Laboratories, Chicago, IL) which recognizes
amino acid residues 70 to 75 of SWISS-PROT Accession No. P17209
(SEQ ID NO:19), anti-TM (Sigma), anti-sarcomeric actin (Sigma),
and anti-glyceraldehyde phosphate dehydrogenase (Cedarline Lab.
Ltd, Canada). Several different anti-TnI antibodies were
utilized: anti-TnI clone 3309 which recognizes amino acid
residues 157 to 192 of SEQ ID NO:11 and clone AM-NI which
recognizes TnI residues 1 to 65 of SEQ ID NO:11 (provided by Dr.
J. Ladenson, Washington University St Louis, Mo.), anti-TnI
clone 10F2 (MAb 10F2) which recognizes amino acid residues 189 to
199 of SEQ ID NO:11 (see epitope map Figure 8 in Van Eyk et al.
1998, *Circ. Res.* 82:261-71), antibody provided by Dr. C. Larue
at Univ. Innsbruck Med. School, Austria, MAb C5 (Research

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Page 3

61
Diagnostics, Flanders, NS), and our anti-TnI peptide (P143T) residues 137 to 148 of SEQ ID NO:11 (MAb E2). The production of the anti-TnI peptide monoclonal antibodies including MAbE2 has been described in Van Eyk et al. 1995 (*Prot. Sci.* 4:781-90). MAb E2 recognizes intact skeletal and cardiac TnI and cardiac TnI peptides containing amino acid residues 136 to 148 of SEQ ID NO:11 (data not shown). As well, anti-TnI antibodies MAb 8I-7 and 3I-35 (both Spectral Diagnostics, Toronto, Canada), and MAb C5 (Research Diagnostics, Flander, NS), which recognize TnI amino acid residues (136 to 147, 188 to 199, and 188 to 199 of SEQ ID NO:11, respectively, see McDonough et al. 1998, *Biophysical J.* 74:A354). Epitope mapping of these various antibodies was carried out by 12% SDS PAGE of intact cardiac TnI and various TnI fragments followed by western blot analysis as outlined above. Bovine cardiac TnI and rabbit skeletal TnI were purified by HPLC (Ingraham et al. 1988, *Biochemistry* 27:5891-98); recombinant rat cardiac TnI fragments 54 to 210, 1 to 188, and 1 to 199 of SEQ ID NO:11 were provided by Dr. A Martin (Univ. Illinois at Chicago, IL; Rarick et al. 1997, *J. Biol. Chem.* 272:26887-92), and the synthetic TnI peptide 96 to 142, which is equivalent to the cardiac peptide residues 129 to 175 of SEQ ID NO:11, was prepared by solid-phase peptide synthesis (Tripet et al. 1997 *J. Mol.*